

SUPPORTING INFORMATION

The CRISPR RNA-guided surveillance complex in *Escherichia coli* accommodates extended RNA spacers

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SUPPLEMENTARY FIGURES

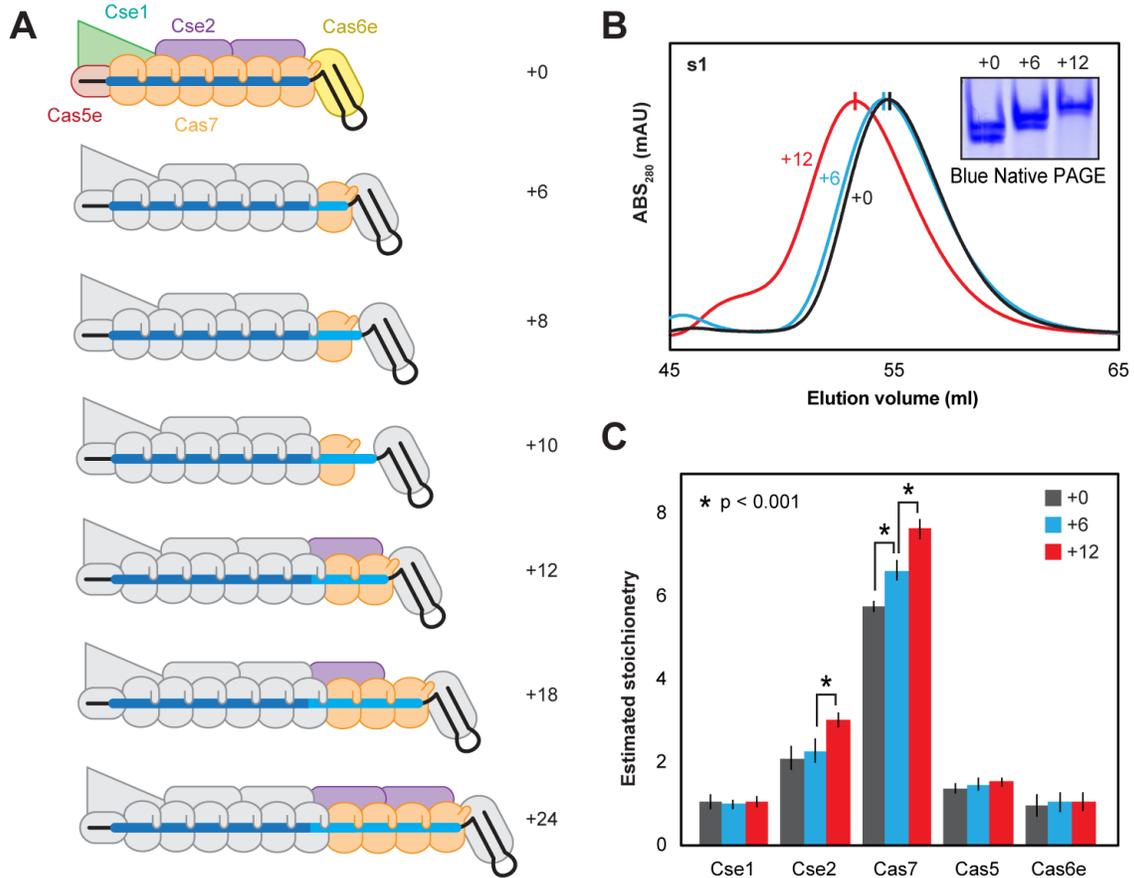


Figure S1. Spacer extension changes protein subunit stoichiometry of *E. coli* Type I-E Cascade. **(A)** Schematics of putative subunit stoichiometry for Cascade complexes bound to a CRISPR RNA with spacer lengths of 32 nts (+0), 38 nts (+6), 40 nts (+8), 42 nts (+10), 44 nts (+12), 50 nts (+18), and +56 nts (+24) depicting how changes in CRISPR RNA spacer length may allow for the addition of Cas7 and Cse2 subunits. **(B)** Gel filtration and Blue Native PAGE (inset) of Cascade complexes (+0), (+6), and (+12) show distinct differences in complex size. **(C)** Band quantification and densitometry analysis of SDS-PAGE assays indicates (+0), (+6) and (+12) complexes consist of the respective subunit stoichiometry depicted in A. The star indicates $p < 0.001$ for $n = 5$. Native Mass Spectrometry also confirmed the predicted stoichiometry for (+0), (+6), and (+12) (see Figure 1).

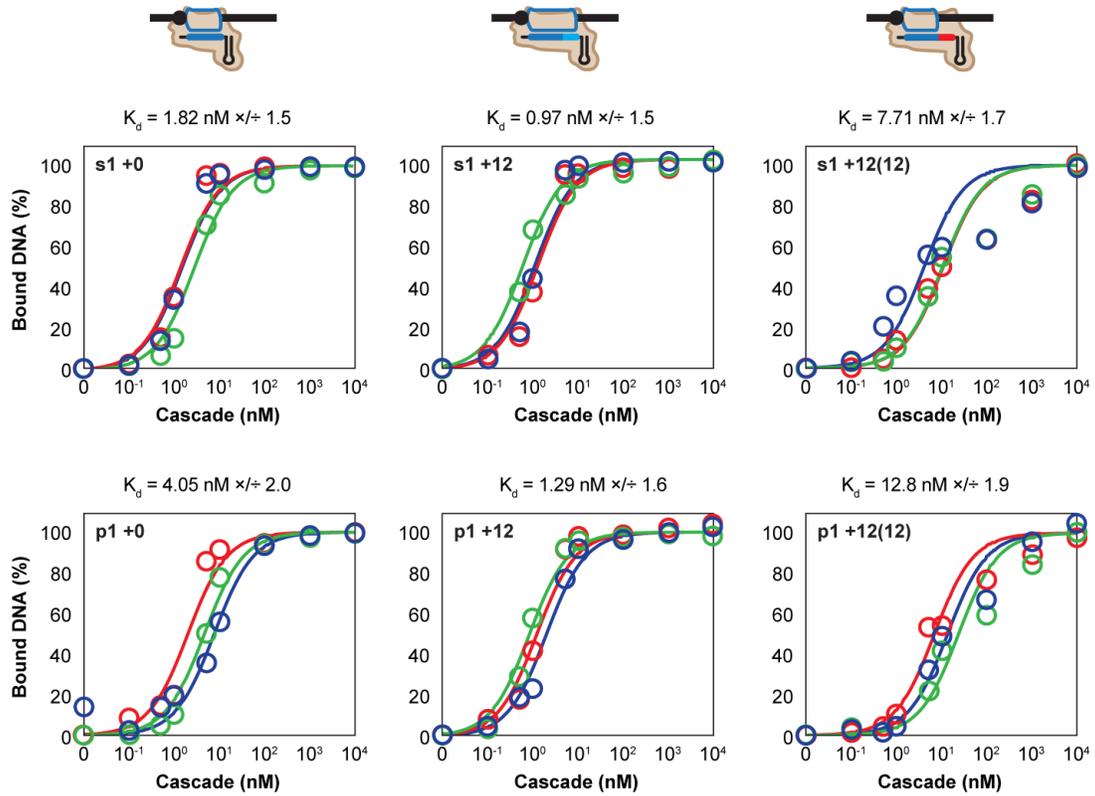


Figure S2. Curve fits for electrophoretic mobility shift assays. The spacer associated with each binding curve is indicated in the upper-left of each panel. Each color represents an independent assay, where circles represent experimental measurements and the curves represent fit Hill equations. The fit values for the apparent dissociation constant (K_d) are shown above each plot.

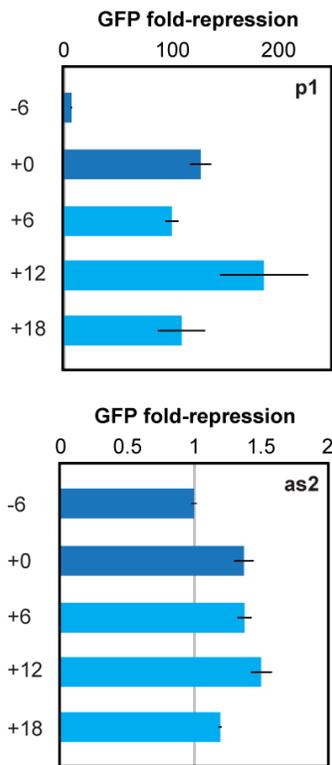


Figure S3. Impact of shortening or extending the spacer on gene silencing by Cascade for the p1 and as2 spacers. Fold-repression is calculated from comparison to a non-targeting control. The gray line indicates a fold-repression value of 1 reflecting no change in GFP fluorescence from the non-targeting control. The depicted values represent the mean and S.E.M. of at least three measurements starting from independent colonies.

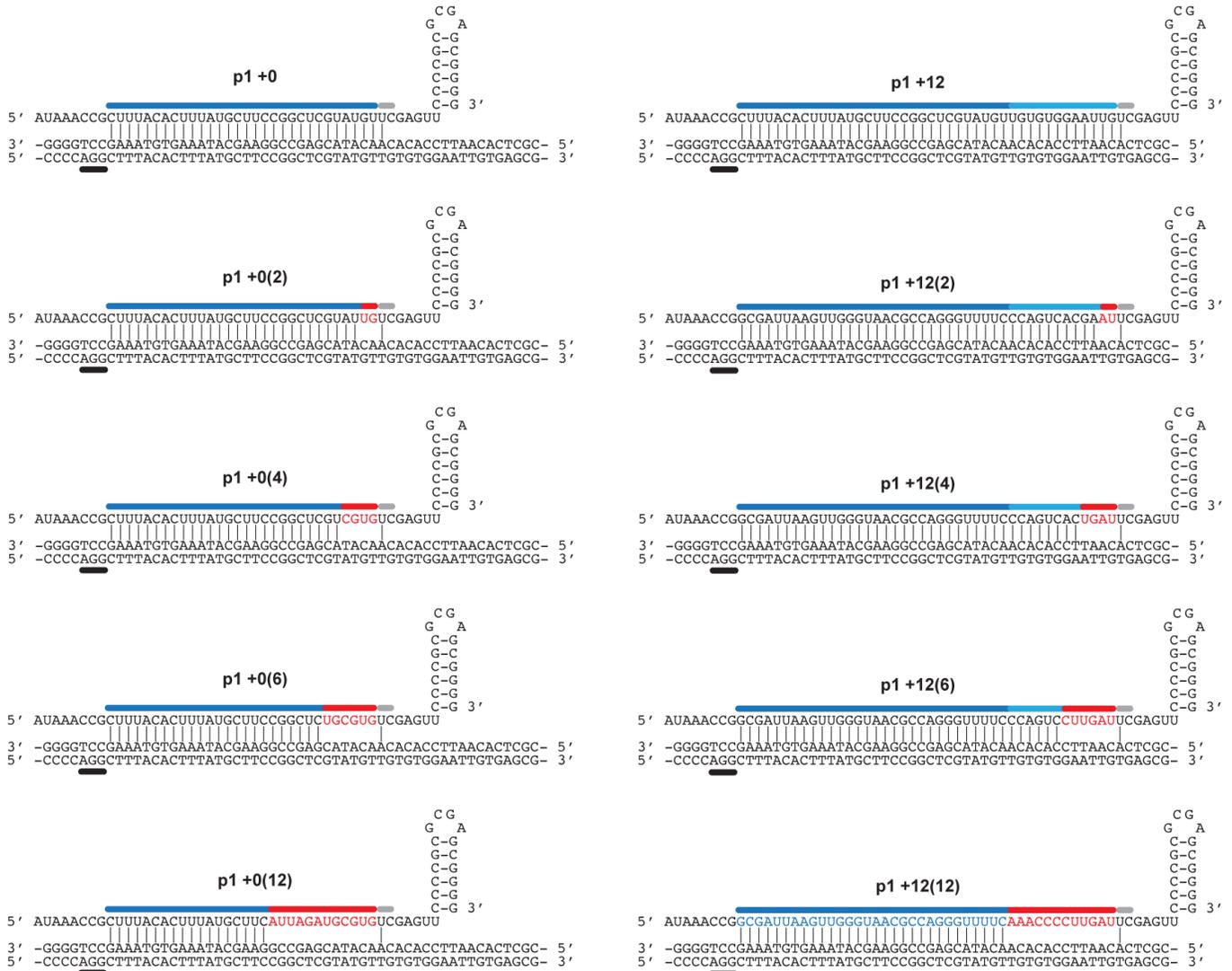
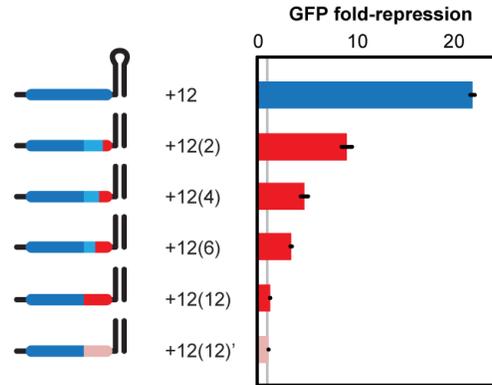
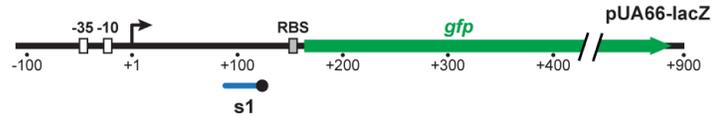


Figure S4. Sequences of p1 spacer variants. The number in parentheses captures the number of mutations made to the 3' end of the +0 or +12 p1 spacer. Black bars indicate the PAM sequence. Dark blue bars indicate the +0 spacer sequence, light blue bars indicate the extended spacer sequence, and red bars indicate mismatched nucleotides.

A



B

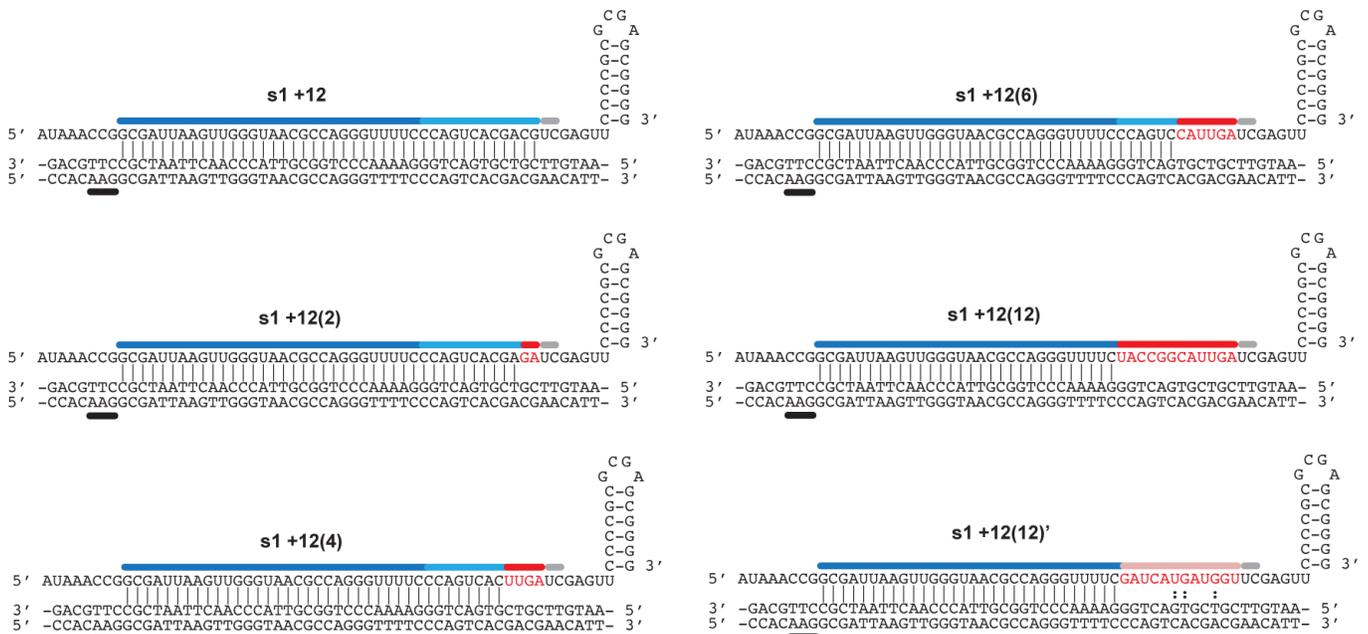


Figure S5. Mismatches on the 3' end of the crRNA reduce gene silencing efficacy with the s1 +12 spacer. **(A)** A schematic showing the location of the s1 target is depicted (top), and a histogram depicting GFP fold-repression for each spacer length (bottom). The gray line indicates a fold-repression value of 1 reflecting no change in GFP fluorescence from the non-targeting control. The depicted values represent the mean and S.E.M. of at least three measurements starting from independent colonies. **(B)** Shown are the crRNA and protospacer sequences for the variants of the s1 +12 spacer. The number in parentheses captures the number of mutations made

to the 3' end of the s1+12 spacer. Black bars indicate the PAM sequence. Dark blue bars indicate the +0 spacer sequence, light blue bars indicate the extended spacer sequence, and red bars indicate mismatched nucleotides.

SUPPLEMENTARY TABLES

Supplementary Table S1. Strains used in this work.

Strains	Genotype	Source	Stock #
BW25113 <i>Δcas3::cat</i>	BW25113 [<i>Δcas3 P_{cse1}</i>]::[<i>cat P_{J23119}</i>]	Supp. Ref. (1)	pCB385
BW25113 <i>Δcas3::cat lacZ⁺</i>	BW25113 [<i>Δcas3 P_{cse1}</i>]::[<i>cat P_{J23119}</i>] <i>lacZ⁺</i>	This study	pCB489
BW25113 <i>Δcas3</i> <i>lacZ⁺</i>	BW25113[<i>Δcas3 P_{cse1}</i>]::[<i>P_{J23119}</i>] <i>lacZ⁺</i>	This study	pCB490
BW25113 <i>ΔCRISPR-Cas</i>	BW25113 [<i>Δcas3-cse1-cse2-cas7-cas5-cas6e-CRISPR1</i>]:: <i>cat</i>	Supp. Ref. (1)	pCB401
MG1655 <i>Δcas3::cat</i>	MG1655 [<i>Δcas3 P_{cse1}</i>]::[<i>cat P_{J23119}</i>]	Supp. Ref. (1)	pCB386

Supplementary Table S2. Plasmids used in this work.

Plasmid	Description	Resistance marker	Source	Stock #
pWUR408	<i>cse1</i> in pRSF-1b, no tags	Kanamycin	Supp. Ref. (2)	pWUR408
pWUR656	<i>cse2</i> (N-terminal Strep II tag) – <i>cas7-cas5-cas6e</i> in pCDF-1b	Streptomycin	Supp. Ref. (3)	pWUR656
pUA66-lacZ	<i>lacZ</i> promoter upstream of GFP	Kanamycin	OpenBiosystems	pCB338
pUA66-xylA	<i>xylA</i> promoter upstream of GFP	Kanamycin	Supp. Ref. (4)	pCB289
pBAD33	L-arabinose-inducible plasmid with <i>araC</i> regulator	Chloramphenicol	Supp. Ref. (5)	pCB442
cBAD33	pBAD33 with constitutive J23108 promoter	Chloramphenicol	This study	pCB491
pCas3	Constitutively expressed <i>cas3</i>	Chloramphenicol	This study	pCB492
pUA66lacZ-NT3PAM-mutant	pUA66lacZ with the PAM of the NT3 spacer mutated	Kanamycin	This study	pCB493
pcrRNA.ind	L-arabinose-inducible CRISPR array with single repeat	Ampicillin	Supp. Ref. (1)	pCB359
pcrRNA.con	Constitutive CRISPR array with single repeat	Ampicillin	Supp. Ref. (1)	pCB379
pcrRNA.ind-p1	pcrRNA.ind with spacer p1	Ampicillin	Supp. Ref. (1) pcrRNA.ind-T2	pCB361
pcrRNA.ind-p1+12	pcrRNA.ind with spacer p1+12	Ampicillin	This study	pCB494
pcrRNA.ind-p2	pcrRNA.ind with spacer p2	Ampicillin	Supp. Ref. (1) pcrRNA.ind-NT2	pCB367
pcrRNA.ind-p2+12	pcrRNA.ind with spacer p2+12	Ampicillin	This study	pCB495
pcrRNA.ind-s1	pcrRNA.ind with spacer s1	Ampicillin	Supp. Ref. (1) pcrRNA.ind-NT3	pCB368
pcrRNA.ind-s1+6	pcrRNA.ind with spacer s1+6	Ampicillin	This study	pCB496

Supplementary Table S2. Continued.

Plasmid	Description	Resistance marker	Source	Stock #
pcrRNA.ind-s1+8	pcrRNA.ind with spacer s1+8	Ampicillin	This study	pCB497
pcrRNA.ind-s1+10	pcrRNA.ind with spacer s1+8	Ampicillin	This study	pCB498
pcrRNA.ind-s1+12	pcrRNA.ind with spacer s1+12	Ampicillin	This study	pCB499
pcrRNA.ind-s1+18	pcrRNA.ind with spacer s1+18	Ampicillin	This study	pCB500
pcrRNA.ind-s1+24	pcrRNA.ind with spacer s1+24	Ampicillin	This study	pCB501
pcrRNA.ind-s2	pcrRNA.ind with spacer s2	Ampicillin	Supp. Ref. (1) pcrRNA.ind-NT4	pCB369
pcrRNA.ind-s2+12	pcrRNA.ind with spacer s2+12	Ampicillin	This study	pCB502
pcrRNA.ind-s3	pcrRNA.ind with spacer s3	Ampicillin	This study	pCB503
pcrRNA.ind-s3+12	pcrRNA.ind with spacer s3+12	Ampicillin	This study	pCB504
pcrRNA.ind-s4	pcrRNA.ind with spacer s4	Ampicillin	This study	pCB505
pcrRNA.ind-s4+12	pcrRNA.ind with spacer s4+12	Ampicillin	This study	pCB506
pcrRNA.ind-as1	pcrRNA.ind with spacer as1	Ampicillin	This study	pCB507
pcrRNA.ind-as1+12	pcrRNA.ind with spacer as1+12	Ampicillin	This study	pCB508
pcrRNA.ind-as2	pcrRNA.ind with spacer as2	Ampicillin	Supp. Ref. (1) pcrRNA.ind-T4	pCB363
pcrRNA.ind-as2+12	pcrRNA.ind with spacer as2+12	Ampicillin	This study	pCB509
pcrRNA.con-p1	pcrRNA.con with spacer p1	Ampicillin	Supp. Ref. (1) pcrRNA.con-T2	pCB380

Supplementary Table S2. Continued.

Plasmid	Description	Resistance marker	Source	Stock #
pcrRNA.con-p1+12	pcrRNA.con with spacer p1+12	Ampicillin	This study	pCB510
pcrRNA.con-p2	pcrRNA.con with spacer p2	Ampicillin	This study	pCB511
pcrRNA.con-p2+12	pcrRNA.con with spacer p2+12	Ampicillin	This study	pCB512
pcrRNA.con-s1	pcrRNA.con with spacer s1	Ampicillin	This study	pCB513
pcrRNA.con-s1+12	pcrRNA.con with spacer s1+12	Ampicillin	This study	pCB514
pcrRNA.con-s2	pcrRNA.con with spacer s2	Ampicillin	This study	pCB515
pcrRNA.con-s2+12	pcrRNA.con with spacer s2+12	Ampicillin	This study	pCB516
pcrRNA.con-as1	pcrRNA.con with spacer as1	Ampicillin	This study	pCB517
pcrRNA.con-as1+12	pcrRNA.con with spacer as1+12	Ampicillin	This study	pCB518
pcrRNA.con-as2	pcrRNA.con with spacer as2	Ampicillin	This study	pCB519
pcrRNA.con-as2+12	pcrRNA.con with spacer as2+12	Ampicillin	This study	pCB520
pcrRNA.ind-p1+12(2)	pcrRNA.ind with spacer p1+12 containing 2 mismatches	Ampicillin	This study	pCB521
pcrRNA.ind-p1+12(4)	pcrRNA.ind with spacer p1+12 containing 4 mismatches	Ampicillin	This study	pCB522
pcrRNA.ind-p1+12(6)	pcrRNA.ind with spacer p1+12 containing 6 mismatches	Ampicillin	This study	pCB523
pcrRNA.ind-p1+12(12)	pcrRNA.ind with spacer p1+12 containing 12 mismatches	Ampicillin	This study	pCB524
pcrRNA.ind-p1(2)	pcrRNA.ind with spacer p1 containing 2 mismatches	Ampicillin	This study	pCB525

Supplementary Table S2. Continued.

Plasmid	Description	Resistance marker	Source	Stock #
pcrRNA.ind-p1(4)	pcrRNA.ind with spacer p1 containing 4 mismatches	Ampicillin	This study	pCB526
pcrRNA.ind-p1(6)	pcrRNA.ind with spacer p1 containing 6 mismatches	Ampicillin	This study	pCB527
pcrRNA.ind-p1(12)	pcrRNA.ind with spacer p1 containing 12 mismatches	Ampicillin	This study	pCB528
pcrRNA.con-p1+12(2)	pcrRNA.con with spacer p1+12 containing 2 mismatches	Ampicillin	This study	pCB529
pcrRNA.con-p1+12(4)	pcrRNA.con with spacer p1+12 containing 4 mismatches	Ampicillin	This study	pCB530
pcrRNA.con-p1+12(6)	pcrRNA.con with spacer p1+12 containing 6 mismatches	Ampicillin	This study	pCB531
pcrRNA.con-p1+12(12)	pcrRNA.con with spacer p1+12 containing 12 mismatches	Ampicillin	This study	pCB532
pcrRNA.con-p1(2)	pcrRNA.con with spacer p1 containing 2 mismatches	Ampicillin	This study	pCB533
pcrRNA.con-p1(4)	pcrRNA.con with spacer p1 containing 4 mismatches	Ampicillin	This study	pCB534
pcrRNA.con-p1(6)	pcrRNA.con with spacer p1 containing 6 mismatches	Ampicillin	This study	pCB535
pcrRNA.con-p1(12)	pcrRNA.con with spacer p1 containing 12 mismatches	Ampicillin	This study	pCB536
pcrRNA.ind-s1+12(2)	pcrRNA.ind with spacer s1+12 containing 2 mismatches	Ampicillin	This study	pCB537
pcrRNA.ind-s1+12(4)	pcrRNA.ind with spacer s1+12 containing 4 mismatches	Ampicillin	This study	pCB538
pcrRNA.ind-s1+12(6)	pcrRNA.ind with spacer s1+12 containing 6 mismatches	Ampicillin	This study	pCB539
pcrRNA.ind-s1+12(12)	pcrRNA.ind with spacer s1+12 containing 12 mismatches	Ampicillin	This study	pCB540
pcrRNA.ind-1+12(12) ⁷	pcrRNA.ind with spacer s1+12 containing 12 mismatches	Ampicillin	This study	pCB541

Supplementary Table S2. Continued.

Plasmid	Description	Resistance marker	Source	Stock #
pcrRNA.ind-s1-6	pcrRNA.con with spacer s1-6	Ampicillin	This study	pCB542
pcrRNA.ind-p1-6	pcrRNA.con with spacer p1-6	Ampicillin	This study	pCB543
pcrRNA.ind-as2-6	pcrRNA.con with spacer as2-6	Ampicillin	This study	pCB544

Supplementary Table S3. Oligonucleotides used in this work.

Name	Sequence
pBad33.J23108.fwd2	tctgacagctagctcagtcctaggtataatgctagcgagct
pBad33.J23108.rev2	cgctagcattatacctaggactgagctagctgtcagatgca
pCas3.for	AAATGGTACCAGGAGGATCAGATGGAACCTTTTAAATATATATGCCATTACTGG
pCas3.rev	TATTTCTAGATTATTTGGGATTTGCAGGGATGAC
NT3-PAM.Q5.for	ACTTAATCGCcgtGCAGCACAGG
NT3-PAM.Q5.rev	TGGGTAACGCCAGGGTTTTTC
p1.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGCTTTACACTTTATGCTTCCGGCTCGT ATGT
p1.rev	TCGAACATACGAGCCGGAAGCATAAAGTGTAAGCGGTTTATCCCCGCTGGCGCGGGGAAC TCGAGGTGGTAC
p1+12.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGCTTTACACTTTATGCTTCCGGCTCGT ATGTTGTGTGGAATTG
p1+12.rev	TCGACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAGCGGTTTATCCCCGCT GGCGCGGGGAACTCGAGGTGGTAC
p2.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGCATAAAGTGTAAGCCTGGGGTGCCT AATG
p2.rev	TCGACATTAGGCACCCCAGGCTTTACACTTTATGCGGTTTATCCCCGCTGGCGCGGGGAAC TCGAGGTGGTAC
p2+12.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGCATAAAGTGTAAGCCTGGGGTGCCT AATGAGTGAGCTAACT
p2+12.rev	TCGAAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCGGTTTATCCCCGCT GGCGCGGGGAACTCGAGGTGGTAC
s1.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGCGATTAAGTTGGGTAACGCCAGGGT TTTC
s1.rev	TCGAGAAAACCTGGCGTTACCCAACCTAATCGCCGGTTTATCCCCGCTGGCGCGGGGAAC TCGAGGTGGTAC

Supplementary Table S3. Continued.

Name	Sequence
s1+6.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACC GGCGATTAAGTTGGGTAACGCCAGGGT TTTCCCAGTC
s1+6.rev	TCGAGACTGGGAAAACCCCTGGCGTTACCCA ACTTAATCGCCGTTTATCCCCGCTGGCGCG GGGAACTCGAGGTGGTAC
s1+8.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACC GGCGATTAAGTTGGGTAACGCCAGGGT TTTCCCAGTCAC
s1+8.rev	TCGAGTGACTGGGAAAACCCCTGGCGTTACCCA ACTTAATCGCCGTTTATCCCCGCTGGCG CGGGGAACTCGAGGTGGTAC
s1+10.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACC GGCGATTAAGTTGGGTAACGCCAGGGT TTTCCCAGTCACGA
s1+10.rev	TCGATCGTGACTGGGAAAACCCCTGGCGTTACCCA ACTTAATCGCCGTTTATCCCCGCTGG CGCGGGGAACTCGAGGTGGTAC
s1+12.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACC GGCGATTAAGTTGGGTAACGCCAGGGT TTTCCCAGTCACGACG
s1+12.rev	TCGACGTGACTGGGAAAACCCCTGGCGTTACCCA ACTTAATCGCCGTTTATCCCCGCT GGCGCGGGGAACTCGAGGTGGTAC
s1+18.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACC GGCGATTAAGTTGGGTAACGCCAGGGT TTTCCCAGTCACGACGTTGTAA
s1+18.rev	TCGATTACAACGTCGTGACTGGGAAAACCCCTGGCGTTACCCA ACTTAATCGCCGTTTATC CCGCTGGCGCGGGGAACTCGAGGTGGTAC
s1+24.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACC GGCGATTAAGTTGGGTAACGCCAGGGT TTTCCCAGTCACGACGTTGTAAAACGAC
s1+24.rev	TCGAGTCGTTTTACAACGTCGTGACTGGGAAAACCCCTGGCGTTACCCA ACTTAATCGCCGG TTTATCCCCGCTGGCGCGGGGAACTCGAGGTGGTAC
s2.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACC GTTCTTCTCCTTTACTCATATGTATAT CTCC
s2.rev	TCGAGGAGATATACATATGAGTAAAGGAGAAGAACGGTTTATCCCCGCTGGCGCGGGGAAAC TCGAGGTGGTAC
s2+12.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACC GTTCTTCTCCTTTACTCATATGTATAT CTCCTTCTTAAATCTA

Supplementary Table S3. Continued.

Name	Sequence
s2+12.rev	TCGATAGATTTAAGAAGGAGATATACATATGAGTAAAGGAGAAGAACGGTTTATCCCCGCTGGCGCGGGGAACCTCGAGGTGGTAC
s3.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGGTTTTCCAGTCACGACGTTGTAAAA CGAC
s3.rev	TCGAGTCGTTTTACAACGTCGTGACTGGGAAAACCGGTTTATCCCCGCTGGCGCGGGGAAC TCGAGGTGGTAC
s3+12.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGGTTTTCCAGTCACGACGTTGTAAAA CGACGGCCAGTGAATC
s3+12.rev	TCGAGATTCACCTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCGGTTTATCCCCGCT GGCGCGGGGAACCTCGAGGTGGTAC
s4.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGTTGGGTAACGCCAGGGTTTTCCAGT CACG
s4.rev	TCGACGTGACTGGGAAAACCCGCGGTTACCCAACCGTTTTATCCCCGCTGGCGCGGGGAAC TCGAGGTGGTAC
s4+12.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGTTGGGTAACGCCAGGGTTTTCCAGT CACGACGTTGTAAAAC
s4+12.rev	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGTTGGGTAACGCCAGGGTTTTCCAGT CACGACGTTGTAAAAC
as1.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGTCGTGACTGGGAAAACCCGCGGTT ACCC
as1.rev	TCGAGGGTAACGCCAGGGTTTTCCAGTCACGACCGGTTTTATCCCCGCTGGCGCGGGGAAC TCGAGGTGGTAC
as1+12.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGTCGTGACTGGGAAAACCCGCGGTT ACCCAACCTAATCGCC
as1+12.rev	TCGAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCAGTCACGACCGGTTTTATCCCCGCT GGCGCGGGGAACCTCGAGGTGGTAC
as2.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGAGATATACATATGAGTAAAGGAGAAG AACT
as2.rev	TCGAAGTTCTTCTCTTTACTCATATGTATATCTCGGTTTTATCCCCGCTGGCGCGGGGAAC TCGAGGTGGTAC
as2+12.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGAGATATACATATGAGTAAAGGAGAAG AACTTTTCACTGGAGT

Supplementary Table S3. Continued.

Name	Sequence
as2+12.rev	TCGAACTCCAGTGAAAAGTTCTTCTCCTTTACTCATATGTATATCTCGGTTTATCCCCGCT GGCGCGGGGAACCTCGAGGTGGTAC
xylA-s1.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGATTTTGAGCCTTCATAACGAACGCGA TCGA
xylA-s1.rev	TCGATCGATCGCGTTCGTTATGAAGGCTCAAATCGGTTTATCCCCGCTGGCGCGGGGAAC TCGAGGTGGTAC
xylA-s1+12.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGATTTTGAGCCTTCATAACGAACGCGA TCGAGCTGGTCAAAT
xylA-s1+12.rev	TCGAATTTTGACCAGCTCGATCGCGTTCGTTATGAAGGCTCAAATCGGTTTATCCCCGCT GGCGCGGGGAACCTCGAGGTGGTAC
p1+12.2mut.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGCTTTACACTTTATGCTTCCGGCTCGT ATGTTGTGTGGAATAT
p1+12.2mut.rev	TCGAATATTCACACAACATACGAGCCGGAAGCATAAAGTGTAAGCGGTTTATCCCCGCT GGCGCGGGGAACCTCGAGGTGGTAC
p1+12.4mut.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGCTTTACACTTTATGCTTCCGGCTCGT ATGTTGTGTGGATGAT
p1+12.4mut.rev	TCGAATCATCCACACAACATACGAGCCGGAAGCATAAAGTGTAAGCGGTTTATCCCCGCT GGCGCGGGGAACCTCGAGGTGGTAC
p1+12.6mut.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGCTTTACACTTTATGCTTCCGGCTCGT ATGTTGTGTGCTTGAT
p1+12.6mut.rev	TCGAATCAAGCACACAACATACGAGCCGGAAGCATAAAGTGTAAGCGGTTTATCCCCGCT GGCGCGGGGAACCTCGAGGTGGTAC
p1+12.12mut.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGCTTTACACTTTATGCTTCCGGCTCGT ATGTAACCCCTTGAT
p1+12.12mut.rev	TCGAATCAAGGGGTTTACATACGAGCCGGAAGCATAAAGTGTAAGCGGTTTATCCCCGCT GGCGCGGGGAACCTCGAGGTGGTAC
p1.2mut.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGCTTTACACTTTATGCTTCCGGCTCGT ATTG
p1.2mut.rev	TCGACAATACGAGCCGGAAGCATAAAGTGTAAGCGGTTTATCCCCGCTGGCGCGGGGAAC TCGAGGTGGTAC
p1.4mut.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGCTTTACACTTTATGCTTCCGGCTCGT CGTG

Supplementary Table S3. Continued.

Name	Sequence
p1.4mut.rev	TCGACACGACGAGCCGGAAGCATAAAGTGTAAGCGGTTTATCCCCGCTGGCGCGGGGAAC TCGAGGTGGTAC
p1.6mut.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGCTTTACACTTTATGCTTCCGGCTCTG CGTG
p1.6mut.rev	TCGACACGCAGAGCCGGAAGCATAAAGTGTAAGCGGTTTATCCCCGCTGGCGCGGGGAAC TCGAGGTGGTAC
p1.12mut.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGCTTTACACTTTATGCTTCATTAGATG CGTG
p1.12mut.rev	TCGACACGCATCTAATGAAGCATAAAGTGTAAGCGGTTTATCCCCGCTGGCGCGGGGAAC TCGAGGTGGTAC
s1+12.2mut.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGGCGATTAAGTTGGGTAACGCCAGGGT TTTCCCAGTCACGAGA
s1+12.2mut.rev	TCGATCTCGTGACTGGGAAAACCCTGGCGTTACCCAACCTAATCGCCGGTTTATCCCCGCT GGCGCGGGGAACTCGAGGTGGTAC
s1+12.4mut.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGGCGATTAAGTTGGGTAACGCCAGGGT TTTCCCAGTCACTTGA
s1+12.4mut.rev	TCGATCAAGTGACTGGGAAAACCCTGGCGTTACCCAACCTAATCGCCGGTTTATCCCCGCT GGCGCGGGGAACTCGAGGTGGTAC
s1+12.6mut.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGGCGATTAAGTTGGGTAACGCCAGGGT TTTCCCAGTCCATTGA
s1+12.6mut.rev	TCGATCAATGGACTGGGAAAACCCTGGCGTTACCCAACCTAATCGCCGGTTTATCCCCGCT GGCGCGGGGAACTCGAGGTGGTAC
s1+12.12mut.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGGCGATTAAGTTGGGTAACGCCAGGGT TTTCTACCGGCATTCA
s1+12.12mut.rev	TCGATGAATGCCGGTAGAAAACCCTGGCGTTACCCAACCTAATCGCCGGTTTATCCCCGCT GGCGCGGGGAACTCGAGGTGGTAC
s1+12.12mut'.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGGCGATTAAGTTGGGTAACGCCAGGGT TTTCGATCATGATGGT
s1+12.12mut'.rev	TCGAACCATCATGATCGAAAACCCTGGCGTTACCCAACCTAATCGCCGGTTTATCCCCGCT GGCGCGGGGAACTCGAGGTGGTAC
s1-6.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGGCGATTAAGTTGGGTAACGCCAGG

Supplementary Table S3. Continued.

Name	Sequence
s1-6.rev	TCGACCTGGCGTTACCCAACTTAATCGCCGGTTTATCCCCGCTGGCGCGGGGAACCTCGAGG TGGTAC
p1-6.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGCTTTACACTTTATGCTTCCGGCTC
p1-6.rev	TCGAGAGCCGGAAGCATAAAGTGTAAGCGTTTATCCCCGCTGGCGCGGGGAACCTCGAGG TGGTAC
as2-6.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGAGATATACATATGAGTAAAGGAGA
as2-6.rev	TCGATCTCCTTTACTCATATGTATATCTCGGTTTATCCCCGCTGGCGCGGGGAACCTCGAGG TGGTAC

Supplementary Table S4. Protospacers targeted in this work.

Spacer name	Target strand	Protospacer sequence
p1-6	AS	AGG CTTTACACTTTATGCTTCCGGCTC
p1	AS	AGG CTTTACACTTTATGCTTCCGGCTCGTATGT
p1+12	AS	AGG CTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTG
p2	S	AAG CATAAAGTGTAAGCCTGGGGTGCCTAATG
p2+12	S	AAG CATAAAGTGTAAGCCTGGGGTGCCTAATGAGTGAGCTAACT
s1-6	S	AAG GCGATTAAGTTGGGTAACGCCAGG
s1	S	AAG GCGATTAAGTTGGGTAACGCCAGGGTTTTTC
s1+6	S	AAG GCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTC
s1+8	S	AAG GCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCAC
s1+10	S	AAG GCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGA
s1+12	S	AAG GCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACG
s1+18	S	AAG GCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAA
s1+24	S	AAG GCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGAC
s2	S	AAG TTCTTCTCCTTTACTCATATGTATATCTCC
s2+12	S	AAG TTCTTCTCCTTTACTCATATGTATATCTCCTTCTTAAATCTA
s3	S	AGG TTTTTCCCAGTCACGACGTTGTAAAACGAC
s3+12	S	AGG TTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAATC
s4	S	AAG TTGGGTAACGCCAGGGTTTTCCCAGTCACG
s4+12	S	AAG TTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAAC
as1	AS	AAC GTCGTGACTGGGAAAACCTGGCGTTACCC
as1+12	AS	AAC GTCGTGACTGGGAAAACCTGGCGTTACCCAACCTAATCGCC
as2-6	AS	AGG AGATATACATATGAGTAAAGGAGA
as2	AS	AGG AGATATACATATGAGTAAAGGAGAAGAACT
as2+12	AS	AGG AGATATACATATGAGTAAAGGAGAAGAACTTTTCACTGGAGT
xylA-s1	S	AGG ATTTTGAGCCTTCATAACGAACGCGATCGA
xylA-s1+12	S	AGG ATTTTGAGCCTTCATAACGAACGCGATCGAGCTGGTCAAAT

- a) Characteristics of the target strand, which is complementary to the spacer: S, sense strand of gene; AS, antisense strand of the gene.
- b) PAMs are in bold red lettering. CRISPR spacers were designed to match the protospacer sequence.

Supplementary Table S5. pUA66 promoter sequences.

Promoter	Sequence
<i>lacZ</i>	<p>CTTTCGTCTTCACACTCGAGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGC GCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCAGGCTTTACACTTTA TGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTACACAGGAA ACAGCTATGACCATGATTACGGATTCACTGGCCGTCGTTTTACAACGTCGTGACTGG GAAAACCCTGGCGTTACCCAACCTAATCGCCTTGCAGCACAGGATCCTCTAGATTTA AGAAGGAGATATACAT</p>
<i>xyIA</i>	<p>CGAGGCCCTTTCGTCTTCACGGTGTAGGGCCTTCTGTAGTTAGAGGACAGTTTTAAT AAGTAACAATCACCGCGATAAACGTAACCAATTTTTAGCAACTAAACAGGGGAAAAC AATTACAGATTTTTATCTTTCGATTACGATTTTTGGTTTATTTCTTGATTTATGACC GAGATCTTACTTTTGTGCGCAATTGTACTTATTGCATTTTTCTCTTCGAGGAATTA CCCAGTTTCATCATTCCATTTTTATTTTGCAGCGAGCGCACACTTGTGAATTATCTC AATAGCAGTGTGAAATAACATAATTGAGCAACTGAAAGGGAGTGCCCAATATTACGA ATCATCCATCACCCGCGGCATTACCTGATTATGGAGTTCAATATGCAAGCCTATTT TGACCAGCTCGATCGCGTTCGTTATGAAGGCTCAAAATCCTCAAACCCGTTAGCATT CCGTCACTACAATCCCGACGAACTGGTGTGGGTAAGCGTATGTAATCTAGATTTAA GAAGGAGAT</p>

- Sequences highlighted in gray are from pUA66, indicating where each promoter was inserted into the plasmid. The underlined and bolded base is the previously mapped transcriptional start site.
- The highlighted yellow text indicates the start codon of *lacZ* contained within the reporter construct.
- The highlighted blue text indicates the region mutated for pUA66*lacZ*-NT3PAM-mutant. Sequence was mutated from CTT to CGT.

Supplementary Table S6. Theoretical and experimentally measured masses of individual Cas protein subunits and Cascade complexes. Discrepancies between theoretical and experimental masses are explained by loss of N-terminal methionines from Cse1, Cse2 with tag, and Cas7 during protein expression.

Cascade Complex	Theoretical Mass (Da)	Experimental Mass (Da)
s1 +0 with 2 tags	405,265.9	405,238.2 ± 45.8
s1 +6 with 2 tags	446,909.1	446,655.1 ± 209.9
s1 +12 with 3 tags	510,232.2	510,127.2 ± 48.9
Cascade Subunit	Theoretical Mass (Da)	Experimental Mass (Da)
Cse1	55901.1	55,841.0 ± 0.1
Cse2 with tag	21,391.5	21,260.3 ± 0.6
Cse2 without tag	19,204.0	19,204.1 ± 0.1
Cas7	40,025.4	39,894.0 ± 1.0
Cas5e	25,208.9	25,208.3 ± 1.2
Cas6e	22,292.9	22,292.0 ± 0.0

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